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(54) Random interesterification of triglyceride fats.

(57) Process for the preparation of a triglyceride mixture with an approximately random distribution of the fatty acid residues over the glyceride positions Sn1, Sn2 and Sn3, by subjecting in the presence of water a triglyceride fat to the activity of a 1,3-specific lipase.
The process is a multi-step process which includes a first step wherein the triglycerides are subjected to enzymatic action in the presence of diglycerides amounting 4-30 wt.% on total fat content and a second step wherein the diglyceride concentration in the reaction mixture is reduced, e.g. by removal of water, optionally after addition of fatty acids.

EP 0 652 289 A1

The present invention deals with an interesterification process of triglyceride fats. More particularly the process concerns an enzymatic interesterification process.

Interesterification processes of triglycerides are directed to an exchange of the fatty acid residues of the triglyceride molecules. In the resulting triglycerides the fatty acid residues have been substituted by different residues, originating from the same or different glyceride molecules or from free fatty acids that may be present in the reaction mixture.

The interesterification process needs a catalyst, which often is an alkali metal hydroxide or an alkali metal alkanolate, such as sodium methanolate. At equilibrium the exchange of fatty acid residues results in a statistically even (random) distribution of the fatty acid residues over the three carbon positions of the glyceride molecule.

Alternatively, a lipase enzyme may be used as a non-chemical catalyst. Some interesterification lipases, the 1,3-specific lipases to which many *Rhizopus* lipases belong, only catalyse reactions on the positions Sn1 and Sn3 of the glyceride molecule. The ester bond on the Sn2 position remains unaffected, with the result that the randomisation process is limited to the terminal positions of the glyceride molecule. It goes without saying that after a interesterification treatment which affects only the terminal fatty acid residues, triglyceride mixtures are obtained of a nature which is quite different from the fully randomised triglycerides known from chemical interesterification. A consequence is that the extensive knowledge on and experience with fully randomised interesterified fats can no longer be used.

Other enzymes, comprising only a few non-specific lipases including *Candida cylindracae* and *Arthrobacter* lipases, are able to catalyse the hydrolysis and subsequent reformation of all three ester bonds with the effect that the randomisation extends also to the Sn2 position (full randomisation).

Processes for enzymatic interesterification processes are described in e.g. WO 91/08676 (KRAFT).

Present consumer preference shifts to food and food ingredients which have not been exposed to chemical treatments. Therefore a general need exists for non-chemical modification processes of triglyceride fats. For interesterification an enzymatic process is preferred, because it does not change the naturalness of the fat product. However, fully randomised fats can only be obtained with non-specific lipases, which generally are either not suited for large scale modification process and/or have not been approved for food manufacture.

On the other hand suitable interesterification enzymes are 1,3-specific lipases but these give no access to fully randomised triglyceride fats.

STATEMENT OF INVENTION

A process has been found for the preparation of a triglyceride mixture with a random distribution of the fatty acid residues over the glyceride positions Sn1, Sn2 and Sn3, by subjecting in the presence of water a triglyceride fat to the activity of a lipase to a randomisation level of at least 50%, characterised in that a 1,3-specific lipase is used.

Preferably, the process is a multi-step process which includes a first step wherein the triglycerides are subjected to enzymatic action in the presence of diglycerides amounting 4-30 wt.% on total fat content and a second step wherein the diglycerides concentration in the reaction mixture is reduced.

The process results in a randomisation at a level which is at least 50%, preferably at least 80% of the theoretically attainable randomisation level.

DETAILS OF THE INVENTION

The randomisation treatment should be carried out with a glyceride mixture which contains elevated levels of diglycerides. Elevated is understood to be in relation to the level normally present in triglyceride fats. The amount should be 4-30 wt.% diglycerides on total fat content.

Such levels of diglycerides are preferably obtained by adjusting the water content to 0.1-1 wt.%, preferably 0.2-0.6 wt.% on total fat content. Water generates the diglyceride *in situ* by fat hydrolysis. However, increased diglyceride levels can also be obtained by direct addition or by enzymatic synthesis *in situ* from glycerol and fatty acids.

The first process step is discontinued when the desired level of randomisation is obtained. The second process step is completed, when the amount of diglycerides is reduced to the desired level, usually <4 wt.%.

Suitable reaction times for the first process step are 10-120 hours, preferably 10-40 hours.

The extent of randomisation resulting from the process is expressed as randomisation level. For defining randomisation level use is made of the occurrence of specific triglycerides in the treated fat, e.g. SSS (S is a saturated long chain fatty acid such as palmitic acid and stearic acid) or the occurrence of a specific fatty acid on Sn2 which occurrences can be easily determined using standard methods. The chosen occurrence is arbitrary with the proviso that it has a value (wt.%) at the start of the process (time = t_0) which is not identical with the random value. The random value can be derived from the fatty acids distribution using standard statistical calculations.

The level (%) of randomisation at time t can be calculated using the formula:

$$\text{Randomisation (\%)} = ((AAA_t - AAA_0) / (AAA_r - AAA_0)) * 100\%.$$

where

AAA_t is actual AAA weight at time t

AAA_0 is actual AAA weight at time 0

AAA_r is calculated AAA weight in a fully randomised triglyceride mixture.

Where AAA is a specific triglyceride class which can be determined by the HPLC/silver phase method.

For establishing the extent of randomisation generally GLC-FAME and 2-position analysis of the initial feed stock and of the reaction mixture is used according to methods to be described later in the examples section. For determining specific triglycerides other methods can be applied e.g. the GLC/carbon number method or the HPLC/silver phase method.

The esterification of glycerides is a process in equilibrium with their hydrolysis, so that removal of one of the reaction products or an increase of the concentration of a reactant shifts the equilibrium to the synthesis of triglycerides under consumption of diglycerides and fatty acids. Therefore the amount of diglycerides may be effectively reduced by removing of the water from the reaction mixture, preferably by evaporating off the water under reduced pressure. The temperature preferably is 60°-80°C. The water evaporation results in low concentrations of diglycerides and fatty acids. The second process step usually takes 5 - 60 hours.

By having increased the concentration of fatty acids when the water is removed the amount of diglycerides can be further reduced.

The enzyme used for carrying out the invention is a 1,3-specific lipase. Suitable lipases may be derived from a micro-organism chosen from the group comprising *Rhizopus*, *Rhizomucor*, *Humicola* or *Pseudomonas*. Although these enzymes affect predominantly the Sn1 and Sn3 positions, some hydrolysis activity on the 2-position may be noticed .

Either the enzyme or an isolate may be used, or a micro-organism containing the enzyme. The enzyme preferably is attached to a carrier such as Duolite™ or Accurel™.

Rhizomucor mienei lipase attached on Duolite™, denoted as SP392, is a suitable catalyst.

The amount of catalyst generally is 1-10 wt.% of the total fat content. The appropriate amount for each system can be easily determined. For SP392 generally 1-5 wt.% is used.

The first process step preferably is carried out at 50-80°C.

The process of the invention can be applied on all kinds of triglycerides, but is most appropriate for triglyceride fats in which there is an imbalance in the distribution of fatty acids over the Sn1,3 and the Sn2 positions by at least one of the oils involved in the interesterification.

Therefore the process is very suitable for palm oil and palm oil fractions which show such imbalance.

The most surprising feature of the invention is that 1,3-specific lipases can be used as dual purpose enzymes.

Depending on reaction parameters, namely the employed level of diglycerides and the reaction time, an interesterified triglyceride results which either is 1,3-randomised or 1,2,3-randomised. The invention provides a non-chemical, technical scale process for the preparation of fully randomised "natural" interesterified triglycerides. These enzymatically prepared fats were earlier not accessible in large amounts. Because the triglyceride composition of such fats are identical with known chemically interesterified fats, protocols for further processing are widely available.

The invention is also embodied in food products in which a fat is incorporated which is obtained by the process of the invention.

DESCRIPTION OF THE FIGURES

Fig. 1 Shows the level (%) of proceeding randomisation of a blend of palm mid fraction and a StOSt rich fat at different times (hours) for a water content of 0.5 wt.% and and an oil/catalyst ratio of 20:1.

Fig. 2 Shows the diglyceride concentration (%) as a function of interesterification time (hours) for a blend of palm mid fraction and a StOSt rich fat with a water content of 0.5 wt.% and an oil/catalyst ratio of 20:1.

Fig. 3 Shows the level (%) of proceeding randomisation of a blend of a PPLa rich fat and a StOSt rich fat at different times (hours) for a water content of 0.5 wt.% and and an oil/catalyst ratio of 20:1.

Fig. 4 Shows the diglyceride concentration (%) as a function of interesterification time (hours) for a blend of a PPLa rich fat and a StOSt rich fat with a water content of 0.5 wt.% and an oil/catalyst ratio of 20:1.

EXAMPLES

General methods

Fame preparation for neutral triglyceride oils

(This procedure is suitable for fats with free fatty acid levels not exceeding 2% w/w).

Weigh 10mg of fat into a 7ml screw-top sample vial.

Add 1ml of Micro Methyl Ester Reagent*, cap and react on a hot block at 60 °C for 5 minutes, with occasional shaking.

Allow to cool and add 2ml of distilled water and then 2ml of Iso-octane with careful agitation. Pipette off top layer into a small auto vial, cap.

This solution is now suitable for injection.

Fame preparation for triglyceride oils containing free fatty acids

Intended for preparation of total FAMEof free fatty acids/combined fatty acids in one.

These samples require a reagent that will methylate FFA and for most cases Saponification followed by reaction with Boron Trifluoride - Methanol reagent is the best choice.

Take 10mg of fat in a 7ml vial suitable for the hot block.

Add 1ml of 2% KOH in methanol solution (0.5M), cap and react on the hot block for 5 minutes at 60 °C, making sure all the fat has dissolved (ie. saponified).

Allow to cool (1 minute) and add 1ml of BF_3/Methanol reagent (fume hood). Replace on the hotblock for 5 minutes.

Allow to cool. Add 2 ml of distilled water and then 2ml Iso-octane, gently invert to extract the FAME into the iso-octane layer. Pipette off upper iso-octane layer into a small auto vial. (If the iso-octane layer appears cloudy, it can be dried over anhydrous sodium sulphate.)

This solution is now suitable for injection.

Lipolysis

About 0.5g of the fat or oil to be analysed is dissolved in dichloromethane (1ml) and is passed through an alumina column (Brockman I alkaline, 2g), with additional solvent washings to remove free fatty acids and partial glycerides. The solvent is removed by evaporation (nitrogen, 50 °C) to recover the triglyceride fraction for further analysis.

Ifthe sample is a liquid or a soft fat, 0.1g of the cleaned up sample is weighed into a vial (25ml). Ifthe sample is a hard fat then 0.05g of a liquid monoacid triglyceride (tri-C9, > 99%purity), is added to 0.05g of the sample in order to aid dissolution. Buffer solution (2ml), and bile salt solution (0.5ml) are added.

This mixture is then placed into a sonic bath set to 40 °C for 5 minutes to ensure complete emulsification. In the case of a particularly hard fat, it may be necessary to carry out the emulsification step at a higher temperature (60 °C). Ifthis is done, then be careful to cool the vial to 40 °C before proceeding to the next stage.

Calcium chloride (0.005g), and lipase (0.02g) is then added and the mixture put back into the 40 °C water bath and shaken for a further 15 minutes.

The length of time required for this stage will depend upon the fat being analysed, and may take some experimentation to determine. As a general rule, the monoglyceride recovery should be between 15 and 25% based on the triglyceride taken.

Hydrochloric acid (1ml of 1M), is then added to stop the action of the enzyme. An internal standard (0.01g of C17 monoglyceride > 99%,accurately known) is then added and mixed in.

This standard will be used to determine monoglyceride recovery following gas chromatography of fatty acid methyl esters from the 2-monoglyceride (C17 peak). The C17 monoglyceride is assumed to have similar extraction behaviour to the 2 - monoglycerides at this stage.

The lipid components are extracted from the mixture and dried by adding diethyl ether (25ml) to the vial, shaking well and transferring the contents to a conical flask, to which anhydrous sodium sulphate (25g) is added. Wash the vial with ether twice more and add to the flask.

After mixing and settling, the ether layer is decanted into a clean vial, and the solvent is blown down (nitrogen, 40 °C).

Dichloromethane (up to 0.5ml) is added to the recovered fat, and this solution is transferred to the preadsorbent strip of a silica TLC plate (0.5mm thickness 20x20cm, Merck S60). A monoglyceride marker is spotted either side of the sample.

The plate is then transferred to a TLC tank. The solvent system used consists of hexane (40ml), diethyl ether (60ml), and formic acid (1ml). The plate is removed when the solvent front is about 1cm from the top of the plate, and is allowed to dry. It is then sprayed with dichlorofluorescein to reveal the monoglyceride band, which is carefully marked.

The monoglyceride band is scraped off of the plate, and the monoglyceride is extracted from the silica, using diethyl ether (5ml, shaking). The silica is removed by filtration, and the solvent blown down as before. The recovered monoglyceride is

* Micro Methyl Ester Reagent: 2 (0.3% KOH in MeOH) : 1 (60/80 Petroleum Spirit) by volume.

then methylated, and analysed (GCFAME/893).

The monoglyceride recovery, and FAME profile at the 2-position can now be calculated from the FAME data.

GC Methods

Column:
30m x 0.53mm DB225 capillary ex J&W Scientific
Injection:
Perkin Elmer PTV on-column injection
Detector:
FID set at 260 °C
Carrier gas:
Helium, typically 50KPa
Solvent retention adjusted to 0.5min
Injector PTV programmed:
0.0min 50 °C
0.5min 250 °C
3.5min 50 °C
Auto injection, typically 0.2µl injection volume of 2mg/ml solutions.

1. Standard Program for Oils and Fats with Fatty Acids Ranging C8:0 to C24:0

Temperature program:
120 °C - 200 °C @ 8 °C/min [WB26]
200 °C - 230 °C @ 2 °C/min

2. Milk Fats and Butyric Acid (C4:0) Content Fatty Acids C4:0 - C24:0

Temperature program:
50 °C - 200 °C @ 10 °C/min [WB22]
200 °C - 230 °C @ 2 °C/min

3. Fast Isothermal Analysis of C14:0 - C24:0

Isothermal oven temperature:
220 °C [WB30]

Example 1

Randomisation of a blend of palm mid fraction and a StOSt rich fat

25 g of a fractionated palm mid fraction were mixed with 25 g of a StOSt-rich fat and 2.5 g of *Mucor miehei* lipase supported on Duolite™. Water was added to the reaction mixture and adjusted to 0.5% (determined by Karl Fischer titration).

In step 1 of the process scheme the reactants were stirred at 70 °C for 24 hours, in a jacketed vessel fitted with a paddle stirrer. Samples were taken at intervals and the SSS-content (weight) determined by silver phase HPLC (Jeffrey B.S.J. JAOCS, Vol 68, No. 5, 1991) and the partial glyceride content determined by straight phase HPLC (Hammond E.W.J., Chromatography, 203, 397, 1981). Step 1 was continued until the formation of a randomised triglyceride mixture has proceeded to 70%, at which stage there were 11% diglycerides in the reaction mixture.

The diglyceride content was reduced in step 2 of the process scheme by keeping the mixture under reduced pressure at 70 °C for 15 hours, resulting in removal of free water. At the end of step 2 the randomisation had proceeded to 82%, whilst the diglyceride content had been reduced to 3%.

The degree of formation of randomised triglyceride as a function of time is illustrated in Fig 1 and the diglyceride content as a function of time in Fig 2.

The level (%) of randomisation at time t was calculated using the formula:

$$\text{Randomisation (\%)} = ((S3_t - S3_0) / (S3_r - S3_0)) * 100\%.$$

where

$S3_t$ is SSS weight at time t

$S3_0$ is SSS weight at time 0

$S3_r$ is calculated SSS weight in a fully randomised triglyceride mixture.

Where SSS is a triglyceride class which can be determined by the HPLC/silver phase method (S representing any long chain saturated fatty acid, in particular palmitic and stearic acids).

The actual SSS content in a fully randomised triglyceride mixture was calculated statistically using the formula:-

$$\text{\% SSS at completion of randomisation} = (\text{wt.\% long chain saturated acids})^3$$

The wt% of saturated acids was determined by FAME GLC by the method given above.

Example 2

Randomisation of a blend of a PPLa rich fat and a StOSt rich fat

7.5 g of a fat rich in the triglyceride PPLa were mixed with 7.5 g of a StOSt-rich fat and 0.75 g of *Mucor miehei* lipase supported on Duolite™. Water was added to the reaction mixture and adjusted to 0.5% (determined by Karl Fischer titration).

The reactants were stirred at 70 °C for 72 hours, in a magnetic stirrer/heater block. Samples taken at intervals and the 2-position of the triglycerides analysed by lipolysis/FAME GLC. The analytical methods used are described under the

heading General methods. The level (%) of randomisation at time t was calculated using the formula:

$$\% \text{ Randomisation} = ((La^2_t - La^2_0) / (La^2_r - La^2_0)) * 100\%$$

where

La^2_t is 2-position lauric acid content at time t

La^2_0 is 2-position lauric acid content at time 0

La^2_r is calculated 2-position lauric acid content in a fully randomised triglyceride mixture.

In a fully randomised product the distribution of lauric acid at the 2 position should be the same as the overall lauric acid distribution.

The degree of formation of randomised triglyceride as a function of time is illustrated in Fig 3 and the diglyceride content as a function of time in Fig. 4.

Claims

1. Process for the preparation of a triglyceride mixture with a random distribution of the fatty acid residues over the glyceride positions Sn1, Sn2 and Sn3, by subjecting in the presence of water a triglyceride fat to the activity of a lipase to a randomisation level of at least 50%, characterised in that a 1,3-specific lipase is used.

2. Process according to claim 1, characterised in that the process is a multi-step process which includes a first step wherein the triglycerides are subjected to enzymatic action in the presence of diglycerides amounting 4-30 wt.% on total fat content and a second step wherein the diglyceride concentration in the reaction mixture is reduced.

3. Process according to claims 1 or 2, characterised in that the triglyceride mixture has a randomisation level of at least 80%.

4. Process according to any one of claims 1-3, characterised in that the triglyceride mixture contains water amounting to 0.1-1 wt.%, preferably 0.2-0.6 wt.% on total fat content.

5. Process according to any one of claims 2-4, characterised in that the second step comprises removal of the water in the reaction mixture resulting in a reduction of the amount of diglycerides.

6. Process according to claim 5, characterised in that the water is removed by evaporation under reduced pressure.

7. Process according to claims 5 or 6, characterised in that the water is removed after a preceding increase of the concentration of fatty acids.

8. Process according to any one of claims 1-7, characterised in that the enzyme is a 1,3-specific lipase derived from *Rhizopus*, *Rhizomucor*, *Humicola* or *Pseudomonas*.

9. Process according to any one of claims 2-8, characterised in that the first process step is carried out at a temperature of 50-80 °C.

10. Process according to any one of claims 1-9, characterised in that the starting triglyceride mixture contains palm oil or a palm oil fraction.

11. Food products in which a fat is incorporated which is obtained by a process according to any one of the preceding claims.

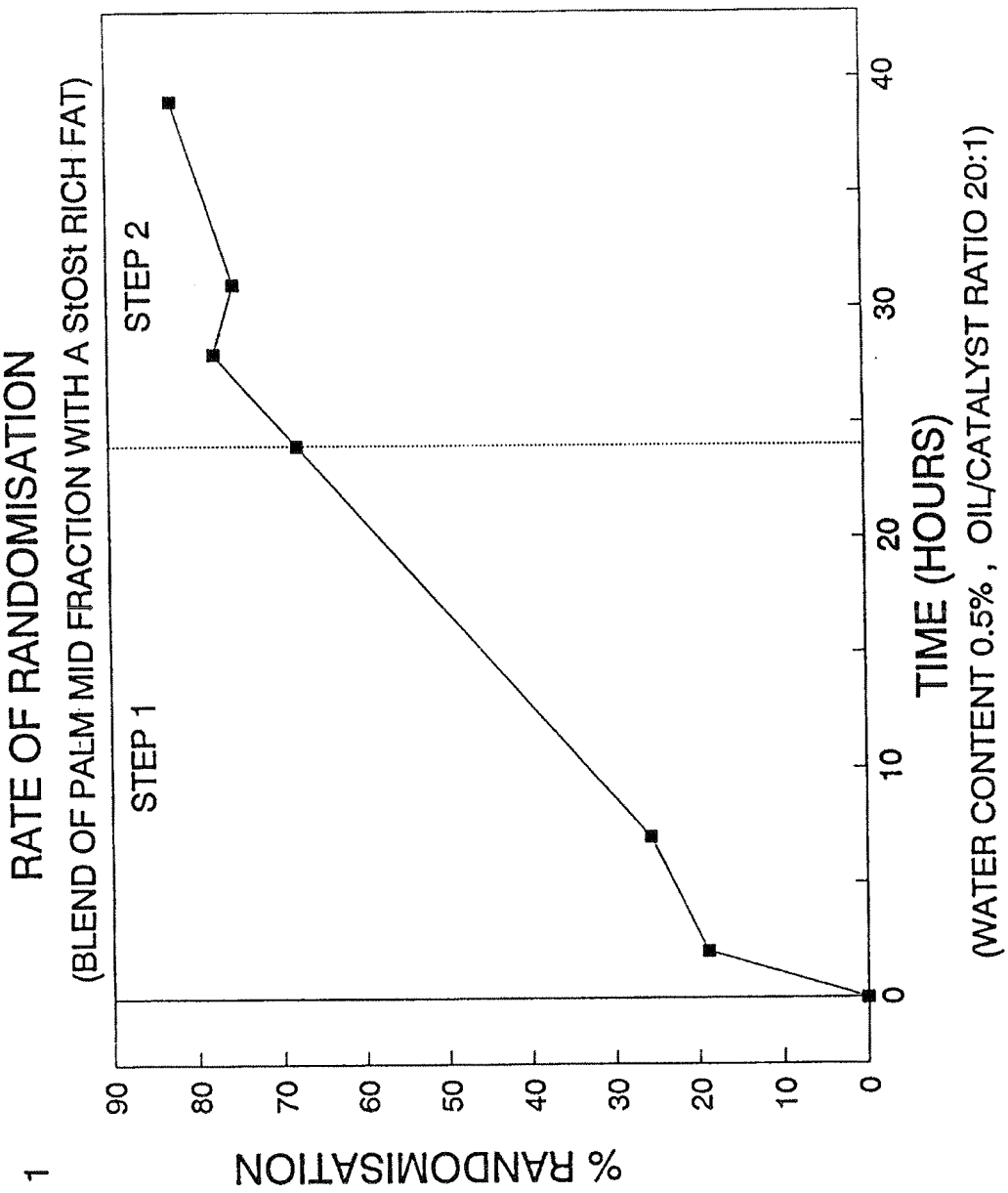
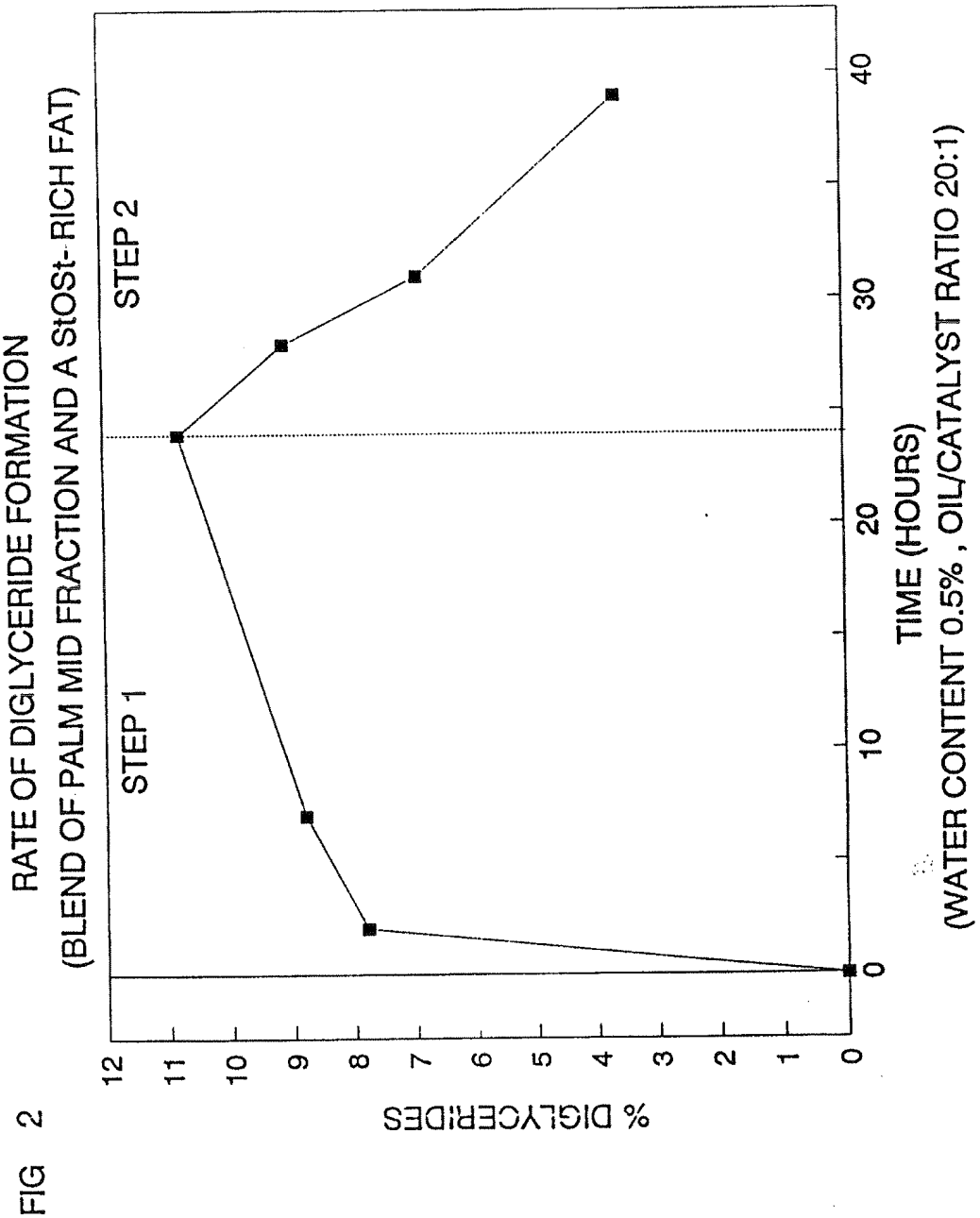

RATE OF RANDOMISATION
(BLEND OF PALM MID FRACTION WITH A StOSt RICH FAT)
STEP 1
STEP 2
% RANDOMISATION
0
10
20
30
40
50
60
70
80
90
TIME (HOURS)
0
10
20
30
40
(WATER CONTENT 0.5% , OIL/CATALYST RATIO 20:1)

FIG 1

FIG 2


RATE OF DIGLYCERIDE FORMATION
(BLEND OF PALM MID FRACTION AND A StOSt- RICH FAT)
STEP 1
STEP 2
% DIGLYCERIDES
0
1
2
3
4
5
6
7
8
9
10
11
12
0
10
20
30
40
TIME (HOURS)
(WATER CONTENT 0.5% , OIL/CATALYST RATIO 20:1)

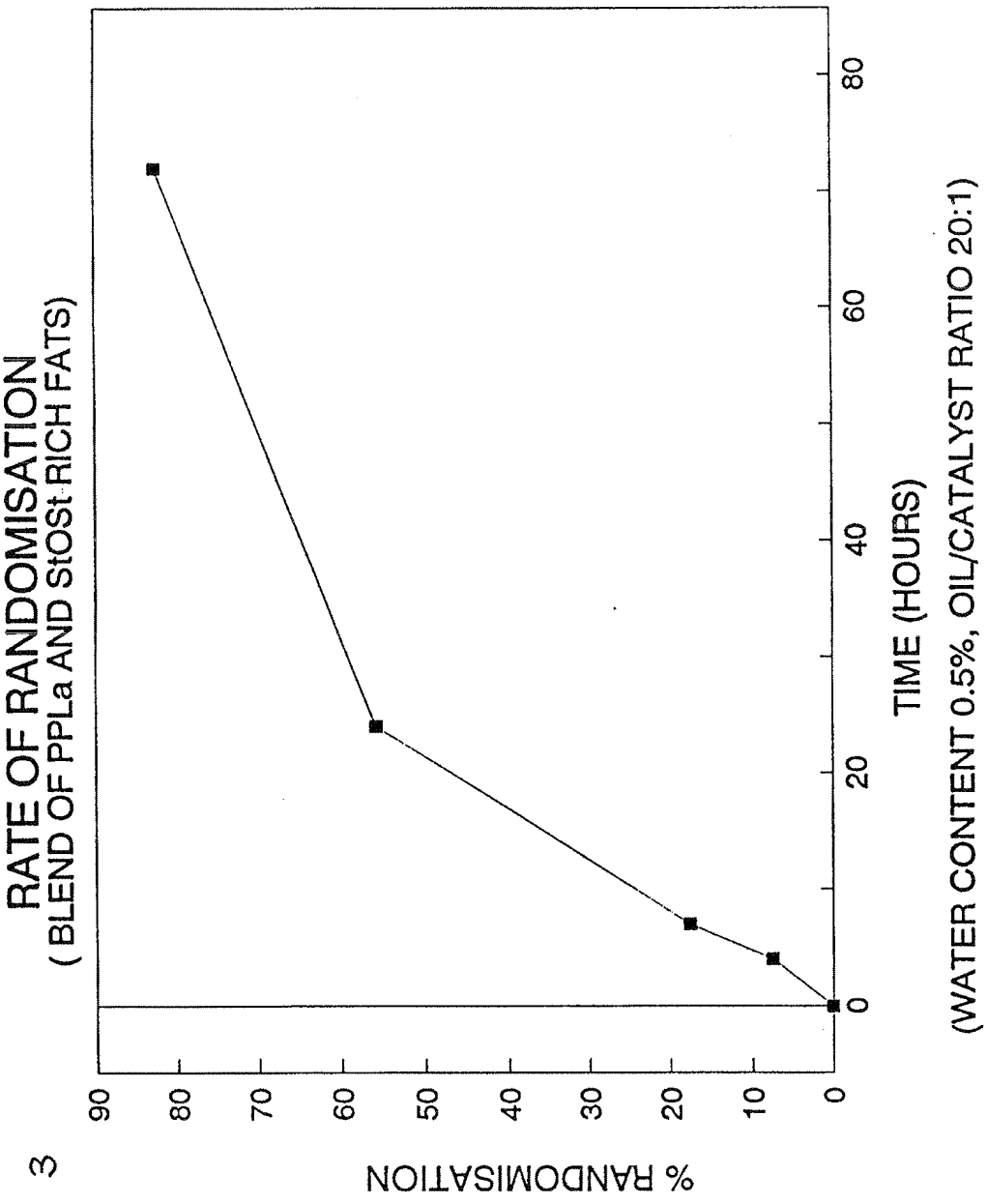

RATE OF RANDOMISATION
(BLEND OF PPLa AND StOSt RICH FATS)
% RANDOMISATION
0
10
20
30
40
50
60
70
80
90
0
20
40
60
80
TIME (HOURS)
(WATER CONTENT 0.5%, OIL/CATALYST RATIO 20:1)

Fig 3

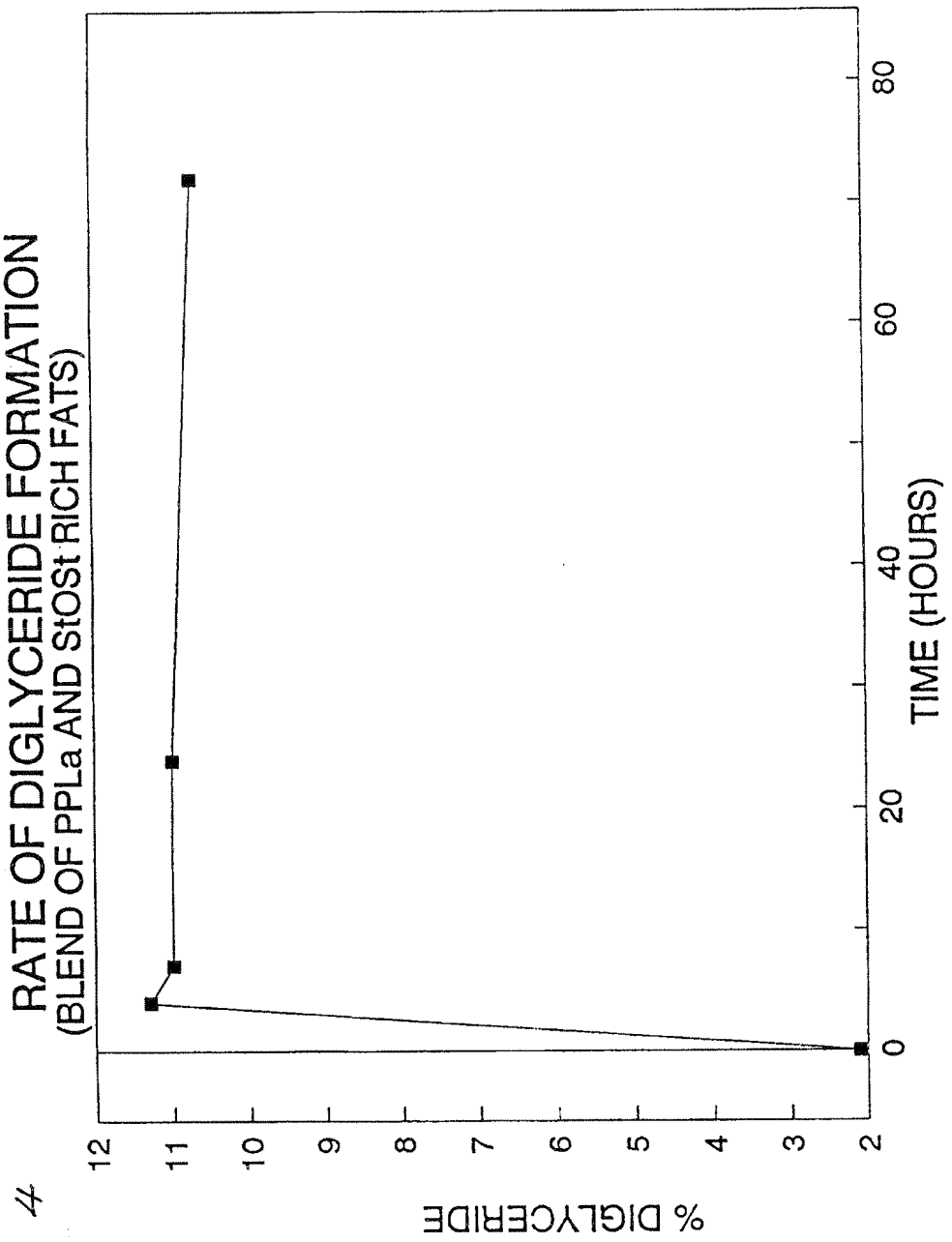

RATE OF DIGLYCERIDE FORMATION
(BLEND OF PPLa AND StOSt RICH FATS)
% DIGLYCERIDE
12
11
10
9
8
7
6
5
4
3
2
0
20
40
60
80
TIME (HOURS)

Fig 4

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Published
With international search report.

(54) Title: PROCESS FOR PREPARING A FAT BLEND AND PLASTIC SPREAD COMPRISING THE FAT BLEND OBTAINED

(57) Abstract

Process for preparing a fat blend comprising partially interesterifying a mixture comprising 20-90 % of a fat (a) and 10-80 % of a fat (b) using an enzyme catalyst to a degree of conversion of 5-95 %, wherein fat (a) is selected from the group consisting of lauric fat, liquid oil and mixtures thereof and fat (b) is a C_{16+} fat having mainly 16 and more carbon atoms in the constituting fatty acid chains thereof and having at least 40 % SAFA. Margarine fat containing such fatblend and spreads made therewith are provided as well. Cost savings are realised and improved product properties can be obtained.

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PROCESS FOR PREPARING A FAT BLEND AND PLASTIC SPREAD COMPRISING THE FAT BLEND OBTAINED

The invention relates to a process for preparing a fat blend and, to plastic spreads containing such fat blends.

For manufacturing plastic W/O emulsion spreads, e.g. margarine, a margarine fat should be used having a well balanced ratio of liquid and solid fats throughout the entire area of use temperatures which usually is from 5°C to about 20°C.

Historically attempts were made to meet this aim by using blends of natural fats having a sufficient solids content, resulting however in products of unsatisfactory spreadability, consistency and mouthfeel. The use of mixtures of fats hardened to different degrees of saturation is another approach, but for nutritional reasons in recent years the desire is expressed that the level of saturated fatty acids (SAFA) of the component triacyl glyceride of the fats should be kept as low as possible. Besides there are consumers who sometimes express some concern about chemically modifying fats e.g. by hydrogenating or hardening, which may result in trans-unsaturation if partial hardening is involved.

Structuring fats for plastic spreads, e.g. W/O emulsion spreads are also obtained by interesterification, resulting in products having considerably improved properties, however at the expense of considerable costs, particularly if enzymatic interesterification is involved.

Enzymatic interesterifcation is nowadays preferred over chemical interesterification because it is effected under much milder conditions, and closely resembles processes occurring widely in nature. It is therefore considered to

be a more "natural" process. However, thusfar costs have been prohibitively high. We have now found a way to substantially reduce the costs. Whereas in some cases products made with fat blends produced according to our cost-reduced process are sensorically undistinguishable from products made with fat blends from conventional processes, we have found that often sensorically perceivable product advantages can be obtained.

The invention provides a process for preparing a fat blend comprising partially interesterifying a mixture comprising 20-90% of a fat (a) and 10-80% of a fat (b) using an enzyme catalyst to a degree of conversion of 5-95%, wherein fat (a) is selected from the group consisting of lauric fat, liquid oil and mixtures thereof and fat (b) is a C_{16+} fat having mainly 16 and more carbon atoms in the constituting fatty acid chains thereof and having at least 40% SAFA. Preferred embodiments of the process are described in claims 2-10. The invention encompasses fat blends obtainable by this process. A preferred embodiment of the fat blend is given in claim 12. The invention also provides margarine fat comprising 8-100% of the present fat blend and 0-92% of liquid oil and/on other fat, said margarine fat having a trans unsaturated fatty acid content of less than 10%; it also provides plastic spread comprising such margarine fat. Preferred embodiments of the spread are given in claims 15-16.

We have extensively studied the mechanisms of the enzymatic interesterification reaction between fats. We found that at high degrees of conversion, all kind of factors increasingly contribute to the cost. Notably enzyme consumption, one of the main cost factors, increases dramatically. In order to obtain valuable fat blends that can provide spreads with good or even improved properties, the choice of fats that are partially interesterified and their mixing ratio are important. We found that when other

fats are used or other mixing ratio's are applied, fat blends are obtained that are less useful than conventional alternatives. Another important aspect affecting costs, we found, is that production capacity can be increased with our process to an extent much higher than proportional to the degree to which the fats were not converted.

For example, we have assessed the relative costs of the enzymatic interesterification as a function of the degree of conversion for a mixture of 35% palmkernel stearine and 65% palm oil stearine, using a 1,3 specific lipase. We obtained the following results.

Degree of conversion (%)	Relative conversion costs (%)
0	0
20	5
40	11
60	20
70	26
80	35
90	50
95	65
100	100

The enzymatic interesterification can be carried out in conventional manner except that the flow rate or the reaction time is adapted to obtain the desired degree of conversion. The choice of enzyme is not critical, any enzyme catalysing interesterification of fatty acid residues of triglycerides can be used. 1,3 specific lipase is preferred. The process is preferably carried out using a continuous design employing e.g. a packed bed reactor containing immobilized enzyme. In a continuous process the interesterification may be controlled by controlling the flow rate or throughput of the reaction mixture with respect to the amount of enzyme. The higher the throughput, the lower the degree of conversion and the lower the price. Alternatively a batch reaction, e.g. in a stirred vessel

may be used. In batch processes the degree of conversion can be controlled by controlling the reaction time.

For a given process design, the flow rate or reaction time required to obtain a pre-selected rate of conversion, varies depending on the prior use of the catalyst. Therefore, the reaction should be monitored frequently. Although the reaction can be carried out in the presence of a solvent, e.g. hexane, preferably such solvent is not used.

The degree of conversion is sometimes also referred to as conversion rate or just conversion, but these expressions are less correct. The degree of conversion indicates the extent to which the reaction has proceeded. For the present purposes it is expressed as

$$\text{Degree of conversion} = (Xt-Xo)/(Xeq-Xo) \times 100\%$$

wherein

X is a measurable property depending on the molecular composition of a triglyceride mixture that reaches its extreme values in the composition prior to the start of the interesterification and in the composition obtained after carrying out the interesterification to completion.

Xo is the value of **X** prior to the interesterification

Xeq is the value of **X** after carrying out the interesterification to completion.

Xt is the value of **X** for the composition for which the degree of conversion is to be determined.

As **X** for example, results from carbon number (CN) analysis or silverphase HPLC analysis can suitably be used. If fat (a) is a lauric fat and fat (b) is a fat rich in C16 and C18 acids, we found CN44+CN46 particularly suitable as **X**. CN44 indicates the percentage of the triglycerides of which the 3 fatty acid chains together have 44 carbon atoms. For

CN46, the 3 fatty acid chains have in total 46 C-atoms. If fat (a) is a liquid oil then silverphase HPLC can suitably be used. Mostly, using the S3 content is appropriate wherein S3 indicates fully saturated triglycerides. If the S3 content in fat (b) is low, then normally the S_2O content, indicating triglycerides with 2 saturated residues and 1 oleic acid residue, will be appropriate. Carbon number and silverphase HPLC analysis are well known techniques. The methods are for example described in EP 78.568 and JAOCS, (1991), 68(5), 289-293, respectively.

The degree of conversion in the present process preferably is 20-93%, more preferably 30-90%, especially 50-90%.

It may perhaps be thought that the same results can be obtained by using in a margarine fat instead of a partially converted mixture, a mixture of a fully converted one and unconverted components in a corresponding ratio. We found however that this is not so. Using the partially converted mixture is more attractive, both for costs reasons and for the resulting product properties.

To obtain optimal results, in the mixture to be partially interesterified, preferably 20-80% of fat (a) and 80-20% of fat (b) are used.

With respect to the choice of fat (a), lauric fat, liquid oil or a mixture thereof may be used. By lauric fat is meant a fat having a content of lauric acid residues of at least 40%, preferably at least 45%. In practice the lauric fats will be coconut oil, palm kernel oil or babassu oil, although in principle some more rare lauric fats can be used as well. For enhancing the structuring effect thereof, in a preferred embodiment, the lauric fats are fractionated and the stearin fraction of those fats as occurring in nature are used in the interesterification.

Although the structuring effect of lauric fats may be increased by hardening, and in particular fully hardening before the interesterification, this option is less preferred than using unhardened lauric fats having regard to naturalness and other considerations mentioned herein.

The terms "fat" and "oil" are used in this specification as synonyms. Fats from which lower melting constituents have been removed will be indicated as "stearin fractions". A stearin fraction for the purpose of this description and claims is defined as a triglyceride mixture or fat blend from which at least 10% of the lower melting constituents have been removed by some kind of fractionation, e.g. dry fractionation or solvent fractionation. Similarly, an olein fraction is a fat from which at least 5% of the higher melting triglycerides have been removed in a fractionation process. A mid fraction is a fat from which, compared with the starting material, at least 3% higher melting triglycerides and at least 10% lower melting triglycerides have been removed in a fractionation process involving at least 2 separation stages.

The term "liquid oil" is used in this specification for glyceride mixtures that are free of solids at 20°C, preferably at 10°C. Preferably, the liquid oil is vegetable oil. Particularly liquid oils containing at least 40% of unsaturated fatty acids (UFA) and in particular of poly unsaturated fatty acids (PUFA), especially linoleic acid, are of importance. Specifically, the liquid oil preferably comprises sunflower oil, soybean oil, rapeseed oil, cottonseed oil, groundnut oil, maize oil, safflower oil, linseed oil, a high oleic acid residue containing variety thereof, e.g. high oleic sunflower oil, high oleic soybean oil or high oleic rapeseed oil, or a mixture of 2 or more of these oils.

Because of the reason expressed before the C16+ fats are preferably unhardened natural fats, however, still containing at least 40% saturated fatty acids (SAFA). Preferably fat (b) comprises 65-100% fatty acids residues having a chain length of 16-24. It is particularly preferred that it comprises 65-100% fatty acid residues with 16-18 carbon atoms in the chain. In a preferred embodiment the fat (b) comprises at least 65% and preferably at least 80% and more preferably at least 90% saturated fatty acids. Suitable examples of fats (b) are high stearic rape seed oil, high stearic sunflower oil, high stearic soybean oil, palm oil, mid fractions or stearin fractions thereof, and mixtures of 2 or more of such fats. However, as a less preferred option fully hardened natural oils having at least 65% C16 and longer chain fatty acids can be used.

If it is desired that the C16+ fat has a high level of saturated fatty acids, stearin fractions of naturally occurring C16+ oils may be used, whereby the above hydrogenation may be obviated.

The terms "hardstock" and "hard fat" herein refer to fatty acid triglycerides of which at least the majority, preferably at least 70%, more preferably at least 90%, of the fatty acids are saturated. Such triglyceride mixtures are solid at ambient temperatures. The hardstock may comprise two or more different hard fats.

The term "structuring fat" is used more generally to indicate fat components that contribute to the structure of a margarine or spread at ambient temperature, typically 20°C, without implying a preference for being highly saturated. Thus, "structuring fat" encompasses hardstock as well as other fat components.

The following 3 embodiments are distinct preferred versions of our process.

A. Fat (a) is lauric fat, preferably palm kernel stearin or possibly fully hardened palm kernel oil.
Fat (b) comprises at least 65% and preferably more saturated fatty acid residues (SAFA). Most preferred is palm stearin with a high melting point, e.g. from solvent fractionation. As an alternative, e.g. fully hardened palm oil can be used. The resulting fat blend is a hardstock, particularly suitable for use together with high contents of liquid oil, preferably at least 75% calculated on the total amount of fat, in making so-called health spreads.

Although in this embodiment the structuring properties of the partially converted mixture may be slightly less pronounced than those of a fully converted mixture, price considerations are favouring the use of such partially converted mixture. Compared with a hardstock of a corresponding mixture of fully interesterified components and not interesterified components, our partially converted fat blend gives organoleptically better results. A partially converted hardstock mixture of a degree of conversion of at least 50% will preferably be used at a level of 8 to 25% on total fat.

Preferably in this embodiment, the degree of conversion is 60-90 %.

The structuring effect of the fat blend obtained by interesterification, when used as a hardstock is optimal if in the interesterification reaction 30-50% preferably 30-40% of the lauric fat and 50-70% preferably 60-70% of the C16+ fat are used.

B. Fat (a) is unhardened lauric fat, preferably palm kernel oil or coconut oil. Fat (b) is an unhardened C16+ fat, preferably having a melting point of less than 53°C, e.g. palm oil, palm stearin from a dry fractionation process and the like. The resulting fat blend is a structuring fat with attractive crystallisation properties and a good N-line. They can be used in high amounts in margarine fat to be used for making the end product. Compared with using components such as palm oil and/or palm stearin together with palm kernel oil without interesterification, several benefits are obtained amongst which is a better solid fat content contribution at different temperatures giving the product better sensoric properties, faster crystallisation allowing higher production speed and reduced risk of separate crystallisation of the palm kernel oil and the palm stearin during production. Such separate crystallisation increases the risk of development of product defects during storage and distribution. For example, products can become substantially softer if they are subjected to temperature cycling. Compared with a fat made by interesterification with complete conversion of such fats, the present fat blend substantially reduces the risks of graininess development in the product. Therefore it can be used in higher amounts. In this embodiment, substantial advantages can already be obtained at very low degrees of conversion, e.g. 20%. Preferably the degree of conversion is 30-90%. Especially the lower degrees of conversion can be obtained at very low costs. These fat blends can be used in margarine fats as structuring components at quite high level, e.g. as much as 50 or 60%, e.g. for making firm wrapper products that must be able to withstand fairly high temperatures.

C. Fat (a) is a liquid oil and fat (b) is a C16+ fat comprising at least 65% SAFA, preferably 80-100%, especially 90-100% SAFA. Without interesterification only little of such hard C16+ fat can be used else it would adversely affect the melt down of the product in the mouth. When applying interesterification to obtain complete conversion, structuring fat can be obtained with a much more desirable contribution to the resulting margarine or spread properties. However, often such structuring fats crystallize very slowly causing problems in production. With such a fat blend partially interesterified according to the present invention, we found faster crystallisation can result. In this embodiment the amount of liquid oil in the mixture to be interesterified preferably is at least 50%, especially 60-90%. If the interesterified mixture is fractionated and the olein is to be used as fat blend in the margarine fat for making spread or margarine, then higher amounts of fat (b) can be included in the mixture to be interesterified, e.g. up to 80%, preferably 30-70%.

A variant on these preferred embodiments that also provides very useful fat blends uses as fat (a) a combination of lauric fat and liquid oil. In this manner the properties of the fat blend can be finetuned to the intended application. In such case, when using in fats (a) and (b) only components that have not been hydrogenated the mixture to be interesterified preferably consists of

20-70% fat (b)
5-60% lauric fat
5-70% liquid oil

The mixture that is partially interesterified preferably consists of fat (a) and fat (b). In such case, by selecting unhardened or carefully and essentially fully hardened products for the interesterification the trans fatty acid

group level in the final fat blend can easily be limited to less than 10% and preferably less than 5% and in particular less than 1% and may even be reduced to 0.2%.

However, other fats, e.g. partially hydrogenated fat may be included in this mixture, e.g. up to 25%. Such inclusion is not preferred, however. If such other fat is included, preferably it is ensured that the trans fatty acid residue content in the final fat blend is still less than 10%, preferably 0-5%.

The process may include fractionating the partially interesterified mixture and recovering a fraction to obtain the fat blend. This can be attractive e.g. if fat (a) comprises liquid oil and an olein fraction is recovered.

The fat blend can be used as such, e.g. in certain cooking applications, e.g. soup making. For such direct application, small amounts of additives may be incorporated in the fat blend, e.g. flavour, colourant, lecithin, etc.

A "margarine fat" is a fat blend which is suitable for use as the sole fat in plastic spreads or in margarine; such a margarine fat usually includes a hardstock or other structuring fat and a liquid oil.

However, for certain applications, fat blend can be used as margarine fat without incorporation of liquid oil, e.g. for bakery applications or spreads for tropical countries if no chilled distribution is used. On the other hand, for making soft spreads packed in tubs, very high contents of liquid oil may be used in the margarine fat. The amount of fat blend in the margarine fat should however be least 8% to obtain benefits of the invention.
Accordingly, the margarine fat comprises 8-100% of our fat blend and 0-92% of liquid oil and/or other fat. The other fat may for example be palm oil, palm olein, palm stearin,

palm mid fraction, coconut oil, palm kernel oil, high stearic acid residue containing varieties of e.g. soybean oil, rapeseed oil, sunflower oil etc., milk fat, and mixtures of two or more of such unhydrogenated oils. Partially or fully hydrogenated oils can be incorporated as well but this is preferably not done. In any case it must be ensured that the trans unsaturated fatty acid content of the margarine fat does not exceed 10%. Preferably, it is 0-6%, especially 0-3%.

The combined amount of fat blend and liquid oil in the margarine fat preferably is 50-100%, more preferably 70-100%, especially 85-100%. The structuring fat of the margarine fat, i.e. all fat other than liquid oil, preferably comprises 30-100%, preferably 50-100% especially 70-100% of fat blend. A combination of two or more different fat blends according to the invention can of course also be used as fat blend in the margarine fat.

The margarine fat can be used as such, optionally in plastified form, e.g. as cooking fat, shortening etc. In such case, the margarine fat may include small amounts of additives e.g. colourant, emulsifier, etc.

According to a preferred embodiment, the margarine fat is used in plastic spread. Such spread comprises a fat phase and an aqueous phase. Either or both of these can constitute a continuous phase. If the product comprises fat only in a dispersed phase, plasticity can be provided by structuring agents in the aqueous phase. Methods to do so are well known in the art. Preferably the spread has a continuous fat phase. The margarine fat is particularly suitable for this type of spread. For microbiological stability of the product it is further preferred that the aqueous phase of the spread constitutes a dispersed phase.

In a particularly preferred embodiment, the spread is a w/o emulsion spread, i.e. comprises a continuous oil phase and a dispersed aqueous phase, the fat phase comprising as margarine fat a fat that includes at least 75% liquid oil and a hardstock that comprises the present fat blend. The amount of hardstock in the margarine fat preferably is 8-25%, especially 12-20%, the balance preferably consisting of liquid oil. The amount of fat blend in the hardstock preferably is 70-100%, more preferably 90-100%, especially 100%.

The fat phase composition may contain apart from margarine fat other materials such as emulsifiers, vitamins, flavour etc. The aqueous phase may comprise apart from water, milk powder, flavour, preservative, gums, etc.

In this specification all parts, proportions and percentages are by weight unless indicated otherwise; the amount of fatty acids in an oil or fat is based on the total amount of fatty acids in said oil or fat and the amount of structuring fat, hardstock and/or hard fat in the fat composition is based on the total weight of said fat composition, unless otherwise stated.

The solid fat content (SFC) in this description and claims is expressed as N-value, essentially as defined in Fette, Seifen, Anstrichmittel 80 180-186 (1978).

For a better understanding of the invention some practical embodiments thereof will be described in the following examples. For manufacturing spreads may be referred to various text books, e.g. The Chemistry and Technology of Edible Oils and Fats and their High Fat Products by G. Hoffmann; Academic Press London 1989, page 319 ff and in particular page 320-321.

The "Stevens" hardness St, expressed in grams, is determined after equilibration for 24 hours at the measuring temperature, using a 4.4 mm ø cylinder in a Stevens-LFRA Texture Analyser (ex Stevens Advanced Weighing Systems, Dunmore, U.K.) load range 1000 g operated "normal" and set at 10 mm penetration depth and 2.0 mm/s penetration rate.

The "thinness" (at 34°C or viscosity in mPa.s at a shear rate of 110 sec^{-1}) is determined using a Viscotester VT181, manufactured by Haake Bros., Berlin. The fat sample is equilibrated for 3 days at 15°C and brought at 34°C in the Viscotester. First the bob of the tester is rotated at position 1 for 1 minute, then switched to position 4 and the reading done 30 seconds thereafter.

EXAMPLE 1

A solvent fractionated palm oil stearin having a palmitic acid content of 76.9% and a dry fractionated palmkernel oil stearin having an unsaturated fatty acid content of 8.1% were blended in a ratio of 50:50 and subsequently interesterified by means of an enzymic catalyst.

The enzymic interesterification reaction was carried out in a labscale packed bed reactor which contained 75 g of the supported enzyme SP392 (commercially available from Novo). The reaction temperature was 70°C and the flow was 50 g/hour. These reaction conditions resulted in a conversion of 97% calculated on the basis of carbon number analysis (Comparison 1).

A second batch of the same composition was enzymically interesterified under the same reaction conditions, with the exception of the flow being 180 g/hour. This resulted in a conversion degree of 74% calculated on the basis of carbon number analysis (Example. 1)

Both reaction products were used as hardstocks and were blended with fully refined sunflower oil in a ratio of 15:85.

From these margarine fats, Comparison 1 and Example 1 respectively, spreads were manufactured. The spreads had the following general composition:

82.8%	margarine fat
0.15%	Admul 6203® (A monodiglyceride emulsifier ex Quest, Naarden, Netherlands)
0.2%	Cetinol® (A lecitin composition ex Unimills, Zwijndrecht, Netherlands)
16.0%	water
0.6%	skimmed milk powder
0.1%	potassium sorbate

The mixtures were processed at laboratory scale through a conventional A-A-C sequence with a throughput of 5.5 kg/hr and a solids content ex C-unit of 6.0%. "A" indicates a surface scraped heat exchanger. "C" indicates a stirred crystalizer. The N-lines of both fats and the hardness and thinness values of the resulting spread products are given in Table 1.

Table 1

Margarine Fat	Comp. 1	Ex. 1
N_{10}	11.0	11.6
N_{20}	4.5	4.0
N_{30}	1.0	2.5
N_{35}	0.4	0.5
Spreads		
St 5	48	47
St 10	38	38
St 15	24	27
St 20	18	16
Thinness 34°C	52	68

Both products showed good spreadability properties, plasticity and exhibited excellent melting behaviour. The thinness values (viscosity at 34°C) were very low compared to corresponding margarines based on interesterifications of hardened components. A panel of experts could not differentiate between the two products although the product with the lowest conversion degree contained more trisaturated triglycerides.

The products showed good stability upon long-term storage and temperature cycling.

EXAMPLE 2

35 parts of palmkernel oil and 65 parts of a palm oil stearin from a multi-stage dry fractionation process were enzymatically interesterified as described in example 1 except that the throughput was adjusted to obtain a degree of conversion of 92%.

For comparison a mixture of 35 parts of palmkernel oil and 65 parts of palm oil stearin from a solvent fractionation process were fully interesterified using a chemical catalyst in a conventional manner (comparison 2).

Margarine fats were prepared by blending 15% of each of these fat blends with 85% sunflower oil.

The N-values of the margarine fats were:

	Ex. 2	Comp. 2
N_{10}	11.6	11.1
N_{20}	7.1	7.4
N_{30}	5.0	4.4
N_{35}	2.1	1.8

Two premixes of fat phase and aqueous phase compositions were prepared as follows:

69.7%	margarine fat
0.1%	monoglyceride
0.2%	lecithin
p.m.	ß-carotene
27.0%	water
1.5%	salt
1.5%	whey powder
p.m.	citric acid to pH 5.2

Fat continuous spreads were produced in a conventional manner using an AAAC sequence. The C-unit was operated at 200 rpm. The product had a temperature of 8.4°C at the end of the line. The fat solid contents at that stage of the process were 8-9% for both formulations. The products were filled into tubs and stored at 5°C.

After one week storage the Stevens values at 5 and 20°C, and the thinness values were measured:

	Ex. 2	Comp. 2
S5	74	73
S20	43	34
Thinness	274	266

The products were evaluated by an experienced panel. Both were found to be good. There were no significant sensoric differences between them.

The products were evaluated again after 9 weeks storage. Both had become more firm. Both were still good products. No significant differences were observed between them.

Both products also responded to temperature cycling in very similar manner.

This example shows that the chemical interesterification and solvent fractionation can be replaced with the more mild and natural processes of partial enzymatic interesterification according to the invention and dry fractionation, without any adverse effect on the nutritional profile of the fatty acid composition or the sensorically perceivable product properties. The Stevens values were overall even slightly higher, which for such product is better, for Ex. 2 relative to Comp. 2.

EXAMPLE 3

Example 2 was repeated except that the throughput was adapted to obtain a degree of conversion of 82%

For comparison, using the same starting materials, the throughput was reduced to obtain a conversion degree of 99% (Comp. 3).

These fatblends were used to prepare margarine fats with the following compositions:

	Ex. 3	Comp. 3
Fatblend 82% conversion	15%	-
Fatblend 99% conversion	-	13%
Palmkernel oil	-	0.7%
Palm oil stearin	-	1.3%
Sunflower oil	85%	85%

The palmkernel oil and the palm oil stearin were from the same batches that were used for preparing the interesterified fatblends. The "average degree of conversion" of the structuring fat of Comp. 3 is even somewhat higher than that of Ex. 3 ([13 x 99 + (0.7 + 1.3)x 0]/ 15 or 86% compared with 82%)

The N-values of the margarine fats were:

	Ex. 3	Comp. 3
N10	8.2	9.6
N20	6.4	7.2
N30	3.9	4.0
N35	2.0	2.7

Spreads were prepared from these fats as described in example 2. The products were evaluated blind by an experienced panel. The panel clearly preferred the products of Ex. 3 compared with Comp. 3 for melting behaviour, taste and overall preference.

EXAMPLES 4 - 8

Example 3 was repeated except that palmkernel oil was replaced with dry fractionated palmkernel stearin. The

trial was done several times using different degrees of conversions.

The premix composition was:

79.55 parts margarine fat
0.15 parts monoglyceride
16.0 parts water
0.6 parts whey powder

The Votator sequence was AAC.

The margarine fat composition and the results obtained were as follows:

	Ex. 4	Ex. 5	Ex. 6	Ex. 7	Ex. 8
Margarine fat					
Fatblend DC* 95%	14	-	-	-	-
79%	-	14	-	-	-
61%	-	-	14	-	-
53%	-	-	-	14	-
43%	-	-	-	-	14
Sunflower oil	86	86	86	86	86
N-values fat					
N10	11.1	10.8	10.4	10.1	10.4
N20	6.6	6.6	7.0	7.0	7.4
N30	3.2	3.9	4.4	4.5	4.9
N35	2.0	2.4	2.7	3.1	3.5
Stevens, Thinness (1 week)					
S5	45	47	47	42	30
S10	46	39	40	35	25
S15	34	32	29	24	22
S20	23	24	22	17	17
Thinness	178	220	280	298	308
Stevens (3 weeks)					
S5	54	51	52	45	32
S10	55	42	43	26	25
S15	35	31	28	21	20
S20	22	21	19	15	16

*Degree of conversion

These results show that the best products are Ex. 4 and Ex. 5. Example 6 still has a suitable texture; the thinness is becoming rather high, but is still acceptable. Ex. 7 and Ex. 8 illustrate that for this type of product degrees of conversion as low as 53 and 43% are less desirable because the thinness continues to increase and the products become rather soft.

EXAMPLES 9 -11

Examples 4, 5 and 6 were repeated. For comparison also a conversion was carried out to 98%. This component was used together with not interesterified components from the same batch. The compositions used were as follows:

	Ex. 9	Ex. 10	Comp. 4	Ex. 11	Comp. 5
Margarine fat					
Fatblend DC*98%	-	-	11.6	-	8.7
95%	14	-	-	-	-
79%	-	14	-	-	-
61%	-	-	-	14	-
palm oil stearin	-	-	1.6	-	3.5
palm kernel stearin	-	-	0.8	-	1.8
sunflower oil	86	86	86	86	86
(Average) DC* of structuring fat(%)	95	79	81	61	61

*Degree of conversion

Whereas there was no very clear difference in the texture and spreadability of Ex. 10 compared with Comp. 4 and of Ex.11 compared with Comp. 5, the samples of the Examples were found to have a better meltdown in the mouth than the corresponding Comparisons.

EXAMPLES 12 - 13

As starting materials, palmkernel oil and a stearin fraction from palm oil obtained by dry fractionation were used. Their fatty acid compositions were measured with FAME GLC analysis. The composition of the fatty acids on the 2-position of the triglycerides was determined as well, using partial hydrolysis of the 1,3 positions and GLC. The following results were obtained:

	Palmkernel oil		Palm stearin	
	Total	2-position	Total	2-position
C6	0.2	-	-	-
C8	3.4	0.6	-	-
C10	3.4	1.6	-	-
C12	47.4	47.6	0.4	0.5
C14	15.7	14.2	1.2	0.8
C16	8.4	4.3	56.4	35.5
C18	2.3	0.7	5.7	2.5
C18:1	16.2	26.4	29.1	49.1
C18:2	2.8	4.5	6.1	11.3
C20	0.1	-	0.4	-
C20:1	0.1	-	0.1	0.1
C22	-	-	0.1	-
Other	-	-	0.5	0.2
Trans	-		-	
SAFA	80.9		64.2	
$C16^+$	29.9		97.9	
C16-18	29.7		97.3	
SMP (°C)	27		51	

A mixture of 50 parts of the palmkernel oil and 50 parts of the palmstearin were enzymatically interesterified in a packed bed reactor using as catalyst the supported 1,3 specific lipase SP 392 (Mucor miehei, immobilised on Duolite, ex Novo Nordisk, Denmark).

The temperature at which the reaction was carried out was 70°C. No solvent was used. The reaction was carried out at several different flow rates and using different amounts of enzym in the packed bed to obtain different degrees of conversion. A smale amount of product was produced at

sufficiently low flow rate to obtain enough sample for analysis of a 100% converted product.

The products obtained as well as the starting materials and the 1:1 mixture prior to the interesterification were analyzed for carbon number. The following results were obtained:

	Palm-kernel	Palm-stearin	1:1 mixture	Ex. 12	Ex. 13	Comp. 6	100% CM#
CN28	0.2	-	0.1	0.1	0.1	0.1	0.1
CN30	1.1	<0.05	0.6	0.5	0.4	0.2	0.2
CN32	6.0	0.1	3.1	2.3	1.6	0.8	0.7
CN34	8.2	0.1	4.2	3.3	2.3	1.2	1.1
CN36	21.0	0.1	10.6	8.5	6.4	3.9	3.7
CN38	15.9	0.1	8.0	7.1	6.2	5.0	4.9
CN40	9.6	0.1	4.9	6.8	8.3	10.0	10.2
CN42	9.6	0.1	4.8	7.6	10.0	13.1	13.4
CN44	7.1	0.2	3.6	6.7	9.8	14.3	14.4
CN46	5.6	0.6	3.6	8.0	12.4	18.1	18.7
CN48	6.6	23.4	14.9	14.5	14.1	13.7	13.7
CN50	2.8	38.7	20.6	17.4	14.5	10.5	10.1
CN52	2.9	27.4	15.0	12.6	10.3	7.2	7.0
CN54	3.3	7.5	5.4	4.3	3.3	1.9	1.8
CN56	0.2	0.5	0.3	0.3	0.2	0.1	0.1
CN58	<0.05	0.1	0.1	0.1	0.1	-	-
CN44+CN46			7.2	14.7	22.2	32.4	33.1
DC*			0%	29%	58%	97%	100%

* DC: Degree of conversion
\# 100% CM: Converted mixture

The CN44 and CN46 obtained for the 100% converted mixture are consistent with an estimate of the CN44 and CN46 calculated from the composition of the starting material using the 1,3 random hypothesis. The table shows that CN44 and CN46 change the most between the unconverted mixture and the 100% converted mixture, while their extremes occur for these two mixtures. Therefore $CN_{44}+CN_{46}$ suitably can be used for calculating the degree of conversion of the products obtained.

The solid fat contents of the products and of the unconverted 1:1 starting mixture were measured. The following results were obtained:

	1:1 mixture	Ex. 12	Ex. 13	Comp. 6
N_{10}	70.6	68.8	69.9	72.8
N_{20}	36.4	34.4	35.1	37.4
N_{30}	15.0	13.1	11.1	9.6
N_{35}	10.3	8.1	5.6	2.1

EXAMPLES 14 - 15

Using the interesterified fatblends of examples 12 and 13 and of comparison 6 and palmkernel oil and palm oil stearin from the same batches as used in these examples, a series of margarine fats were produced by mixing the components together with sunflower oil in the following proportions:

	comp. 7	Ex. 14	Ex. 15	comp. 8	comp. 9
Fatblend comp. 6	50	-	-	15.5	-
Fatblend ex 13	-	50	-	-	-
Fatblend ex 12	-	-	50	-	-
Palmkernel oil	-	-	-	17.3	25
Palm oil stearin	-	-	-	17.3	25
Sunflower oil	50	50	50	50	50
DC* (%)	97	58	29	30	0

*DC = (Average) degree of conversion of structuring fat.

The N-values of the margarine fats were:

	Comp. 7	Ex. 14	Ex. 15	Comp. 8	Comp. 9
N_{10}	27.9	26.3	25.5	27.4	28.2
N_{20}	11.5	10.5	10.4	10.9	11.9
N_{30}	1.7	3.5	4.5	5.6	7.6
N_{35}	0.2	1.6	2.9	4.2	5.4

Using these margarine fats, premixes were produced of fat phase and aqueous phase compositions as follows:

fatphase composition

76.60 parts margarine fat
0.10 parts monoglyceride
0.15 parts lecithin
0.15 parts ß-carotene solution

aqueous phase composition

18.90 parts water
0.30 parts salt
0.70 parts whey powder
0.10 parts potassium sorbate
p.m. citric acid to pH 4.8

Margarines were produced using an A-C-A-C sequence. The A-units were operated at 800 rpm, the C-units at 150 rpm. The premix temperature was 55°C. The temperature after the second A-unit was 7°C in all cases, the temperature after the last C-unit was 9.5°C. At this stage of the process the formulations all contained 16-17% crystallized fat. The products were filled into tubs and stored at 10°C.

The products were evaluated blind by an experienced panel after one week storage, after the samples had been conditioned at 15°C for 24 hours. The product of comparison 9 was rejected. Its melting in the mouth was very poor and its spreadability also was not good. The other 4 products

were judged to be acceptable but of these, comparison 8 was the least liked because it had less good melting behaviour. Comparison 7 melted very quickly in the mouth, which was not appreciated by some of the panellists. Overall the sample of Ex. 14 was liked the best. The findings regarding melting in the mouth were in line with expectations in view of the N-values of the margarine fats. Indeed, we were surprised by the big difference in N_{30} and N_{35} between Example 15 and Comparison 8. The samples were also characterised by their Stevens values and thinness values.

A parallel set of samples from the same production runs were tested for stability to temperature cycling. These samples were stored first 2 days at 10°C, then 12 hours at 5°C and 12 hours at 20°C, this cycle of 5°C and 20°C was repeated 2 more times and then the samples were stored again at 10°C for 2 days. For these samples also Stevens and thinness values were measured. All samples were conditioned 24 hours at the measuring temperature for the Stevens values and at 15°C for the thinness values measurements. The following results were obtained:

	Comp. 7		Ex. 14		Ex. 15		Comp. 8		Comp. 9	
	N*	C*	N	C	N	C	N	C	N	C
S_5	156	175	203	184	294	189	358	213	461	346
S_{10}	159	94	147	107	195	109	231	120	328	97
S_{15}	62	54	63	47	72	49	69	60	132	99
S_{20}	31	33	32	28	29	30	31	32	45	57
Thinness	78	68	184	114	262	222	336	364	480	480

N*: Stored at 10°C
C*: Samples subjected to temperature cycling

The results of the N-samples are consistent with the finding of the panel. The thinness of Comp. 7 is very low while those of Comps. 8 and 9 are high. The Stevens values

for Comp. 9 are quite high, adversely affecting spreadability.

Upon temperature cycling, the thinness of Comp. 7 becomes even slightly lower. Those of Exs. 14 and 15 improve somewhat, while Comp. 8 deteriorates. The thinness of Comp. 9 remains unacceptably high. The Stevens values show that upon temperature cycling especially the products of Comps. 8 and 9 become very much softer at the lower temperatures. Such softening is undesirable because it causes the products to change perceivably after the consumer has left them a few times at the breakfast table for some time. The softening for the other products is less upon temperature cycling.

These trials show that with partial interesterification products can be obtained of comparable quality as when using complete conversion. For some customers the partially converted products may even be preferable. Comparison of Ex. 15 with comparison 9 shows that as little as interesterification to a degree of conversion of 29% dramatically improves the product properties. Comparison of Ex. 15 with Comp. 8 further shows that partial conversion gives better results than using a mixture of fully converted and non-converted components having overall the same average degree of conversion.

CLAIMS

1. Process for preparing a fat blend comprising partially interesterifying a mixture comprising 20-90% of a fat (a) and 10-80% of a fat (b) using an enzyme catalyst to a degree of conversion of 5-95%, wherein fat (a) is selected from the group consisting of lauric fat, liquid oil and mixtures thereof and fat (b) is a C_{16+} fat having mainly 16 and more carbon atoms in the constituting fatty acid chains thereof and having at least 40% SAFA.

2. Process according to claim 1 wherein the degree of conversion is 20-93%, preferably 30-90%, more preferably 50-90%.

3. Process according to claim 1 or claim 2 wherein the C_{16+} fat (b) comprises 65-100% fatty acids having a chain length of 16-24, preferably 16-18 carbon atoms.

4. Process according to claims 1-3, wherein the lauric fat is unhardened.

5. Process according to claims 1-4, wherein the lauric fat is a stearin fraction of a fat occurring in nature.

6. Process according to claims 1-5 wherein the C_{16+} fat (b) is a fully hardened natural oil.

7. Process according to claims 1-5, wherein the C_{16+} fat (b) is a natural oil, a stearin fraction of a natural oil or a mixture thereof.

8. Process according to claims 1-7, the mixture comprising 30-50%, preferably 30-40% of lauric fat and 50-70%, preferably 60-70% of the C_{16+} fat (b).

9. Process according to claims 1-8 wherein the liquid oil comprises sunflower oil, soybean oil, rapeseed oil, cottonseed oil, groundnut oil, maize oil, safflower oil, linseed oil, a high oleic acid containing variety thereof, or a mixture of two or more thereof.

10. Process according to claims 1-9 wherein the partially interesterified mixture is fractionated and a fraction is recovered

11. Fat blend obtainable by the process of any one of claims 1-10.

12. Fat blend according to claim 11, comprising less than 10% and preferably less than 5% and in particular less than 1% trans unsaturated fatty acid groups.

13. Margarine fat comprising 8-100% of fat blend according to claims 11-12 and 0-92% of liquid oil and/or other fat, said margarine fat having a trans unsaturated fatty acid content of less than 10%.

14. Plastic spread comprising a margarine fat according to claim 13.

15. Spread according to claim 14 comprising a continuous fat phase and dispersed aqueous phase.

16. Spread according to claim 15 comprising at least 15% of aqueous phase, the margarine fat comprising at least 75% liquid oil and a hard stock comprising the fat blend of claims 11-12.

INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A23D9/00 A23D7/00 C11C3/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A23D C11C C12P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO,A,93 24017 (UNILEVER) 9 December 1993 see page 8, line 25 - page 9, line 31 see page 10, line 5 - line 22 see page 17, line 32 - line 36 see claims 1-5,23,37,42	1,11
Y	EP,A,0 034 065 (UNILEVER) 19 August 1981 see column 8, paragraph 2 - column 9, paragraph 3 see claim 7	1,11
A	WO,A,94 10326 (LODERS CROKLAAN) 11 May 1994 see claims 1,6,9	1,2,11

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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